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# Allogeneic Stem Cell Transplantation for Aplastic Anemia

*Philippe Armand, Joseph H. Antin*

Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts

Correspondence and reprint requests: Philippe Armand, MD, PhD, Dana-Farber Cancer Institute, 44 Binney Street, Boston MA 02115 (e-mail: parmand@partners.org).

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## ABSTRACT

**Aplastic anemia encompasses a heterogeneous group of diseases with distinct pathophysiologies and a common clinical endpoint of marrow failure. Patients with severe aplastic anemia can be treated with immunosuppressive therapy (IST) or hematopoietic stem cell transplantation (HSCT). Over the last 30 years, advances in both treatment modalities have significantly improved the prognosis for this disease; yet this evolution complicates the central therapeutic question in aplastic anemia: which patients should receive IST and which ones should receive HSCT as front-line therapy? In this review, we describe the major improvements that have occurred in transplantation for aplastic anemia in the last 3 decades. We then outline a framework for deciding which patients should be considered for upfront transplantation.**


## KEY WORDS

Aplastic anemia • Allogeneic stem cell transplantation • Immunosuppressive therapy

## INTRODUCTION

Aplastic anemia (AA) was first described in a pregnant woman by Paul Ehrlich in 1888 [1]. The term now refers to a clinical syndrome of bone marrow hypocellularity accompanied by peripheral pancytopenia. Despite this apparent simplicity, AA is a heterogeneous disease, and is perhaps more appropriately viewed as the common pathologic end point of a variety of possible injuries to the hematopoietic system. Disorders leading to aplasia can be inherited or acquired, with distinct pathophysiologies. The inherited marrow failure syndromes include Fanconi anemia, dyskeratosis congenita, Diamond-Blackfan anemia, and Schwachman-Diamond syndrome. Our understanding of the molecular basis for those disorders has progressed greatly in recent years. The underlying defects have now been mapped to DNA damage repair mechanisms, telomerase regulation, and ribosomal function [2]. In contrast, the proximal cause of acquired aplastic anemia appears in most cases to be not an intrinsic defect in the hematopoietic stem cell, but an immune attack on the hematopoietic system [3]. In some cases, the aplasia can be traced to a definite trigger such as a drug or toxin exposure (two notorious examples are benzene and chloramphenicol), seronegative viral hepatitis, or associated with other conditions such as paroxysmal nocturnal hemoglobinuria (PNH), pregnancy, or eosinophilic fasciitis [4]. A large epidemiologic study from Thailand has also implicated exposure to pesticides and animal fertilizer [5]. In the majority of cases, however, no trigger can be identified for acquired AA. Moreover, even when the trigger is known, the mechanism leading from the causative agent to aplasia is unclear, although the end result is either stem cell loss (often irreversible) or immune-mediated stem cell destruction (which may be reversible by immunosuppression or withdrawal of the offending agent).

For patients with AA requiring treatment, 2 therapeutic modalities can be used: immunosuppressive therapy (IST) or allogeneic hematopoietic stem cell transplantation (HSCT). Those modalities have a different mechanism of action and very different toxicity and efficacy profiles (summarized in Table 1), which must be taken into account when approaching treatment decisions for a patient who is potentially eligible for both. In this review, we summarize the major advances in the practice of stem cell transplantation for AA, and use this as a basis for

**Table 1.** *A Comparison of the Salient Features of Immunosuppressive Therapy (IST) and Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)*

| | IST | HSCT |
|---|---|---|
| Patient exclusions | Severe comorbidities | Advanced age<br>Comorbidities<br>Donor unavailability |
| Relapse risk | 35%-45% | 5%-20%* |
| Relapse pattern | Anytime | Early* |
| Early toxicity | Mild (drug adverse effects) | Conditioning toxicity (5%-10%)<br>Acute GVHD (10%-50%) |
| Late toxicity | PNH (15%-20%)<br>MDS/AML (10%-20%)<br>Solid tumors (2%) | Chronic GVHD (20%-50%)<br>Solid tumors (2%)<br>Other† |

PNH indicates paroxysmal nocturnal hemoglobinuria; MDS, myelodysplastic syndrome; AML, acute myelogenous leukemia; GVHD, graft-versus-host disease.

*In the transplant setting, relapse refers to graft failure, which usually occurs early.

†Including growth retardation, cataracts, hypothyroidism, and infertility.

See text for references.

discussing the choice of first-line therapy for patients with severe AA. Several excellent reviews have been previously written on the topic [3,6-9]. For reference, results of selected studies of transplantation and immunosuppressive therapy are summarized in Tables 2 and 3, respectively.

## ADVANCES IN ALLOGENEIC TRANSPLANTATION

### First Attempts

The first (unsuccessful) reported attempt to intravenously infuse allogeneic marrow for a patient with AA dates back to 1939 [10]; one year later, Morrison and Samwick [11] performed a sibling marrow transplant through an intrasternal infusion on a patient in New York. The outcome was apparently successful, although the patient may not, in fact, have truly suffered from AA. The first successful transplant using a syngeneic donor was reported in 1961 [12], and the first successful matched related donor (MRD) transplant in 1972 [13]. Four years later, a randomized prospective trial from Seattle showed a survival benefit of MRD HSCT over standard of care (supportive transfusions or androgen treatment) [14], proving the usefulness of transplantation in this disease.

### The Importance of Graft Failure

Graft failure (GF) is a central problem in transplantation for AA, occurring more frequently than in other HSCT indications. This likely occurs for two reasons: first, most conditioning regimens for AA are nonmyeloablative. In fact, the observation of autologous recovery with concomitant cure of the aplasia after attempted transplantation [15] is what prompted the development of high-dose cyclophosphamide without grafting, pioneered by Brodsky and colleagues [16]. Second, and more importantly, the antihematopoietic immune activity in the host can reject the graft by the same mechanism that it attacked the recipient's stem cells in the first place. In the initial experience with MRD transplants in the 1970s, using cyclophosphamide (Cy) alone for conditioning and methotrexate alone for graft-versus-host disease (GVHD) prophylaxis, the incidence of GF with MRD transplants was up to 30% [17,18]. As would be predicted on pathophysiologic grounds, intensification of the conditioning regimen or of the posttransplantation immunosuppression can reduce this risk, but may

**Table 2.** *Results of Selected Studies of Stem Cell Transplantation for Severe Aplastic Anemia*

| Study | N* | Median Age (Range) | Conditioning | GVHD Prophylaxis | Match | GF | Survival |
|---|---|---|---|---|---|---|---|
| Kahl et al [27] | 81 | 25 (2-63) | Cy + ATG | CSA + Mtx | MRD | 4% | 15 y OS = 88% |
| Resnick et al [36] | 13 | 20 (9-55) | Cy + Flu + ATG | CSA | MRD | 0% | 5 y OS = 84% |
| Gupta et al [40] | 33 | 16 (4-45) | Cy + Alemtuzumab | CSA | MRD | 24% | 5 y OS = 81% |
| Kim et al [103] | 113 | 28 (16-50) | Cy + ATG + Procarbazine | CSA + Mtx† | MRD | 15% | 6 y OS = 89% |
| Passweg et al [99]‡ | 181 | 16 (1-55) | Varied | Varied | MUD (serologic typing) | 17% | 5 y OS = 39% |
| Deeg et al [31]‡ | 62 | 18 (1-53)¶ | Cy + ATG + TBI | CSA + Mtx | MUD (molecular typing) | 1% | 5 y OS = 61% |
| Bacigalupo et al [34]‡ | 33 | 14 (3-37)¶ | Cy + ATG + Flu | CSA + Mtx | MUD (molecular typing) | 18% | 6 y OS = 73%¶ |
| Kojima et al [30]‡ | 79 | 17 (1-46)¶ | Varied | CSA + Mtx§ | MUD (molecular typing) | 8% | 5 y OS = 60% |

GVHD indicates graft-versus-host disease; GF, graft failure; Cy, cyclophosphamide; ATG, antithymocyte globulin; Flu, fludarabine; TBI, total-body irradiation; CSA, cyclosporine; Mtx, methotrexate; MMF, mycophenolate mofetil; MRD, matched related donor; MUD, matched unrelated donor; OS, overall survival.

*Number of patients.

†Twenty-one percent of patients received a T cell-depleted graft.

‡Published results include some patients transplanted from mismatched donors; except where indicated, only the results for the matched unrelated patients are included in this table.

¶Includes mismatched unrelated patients.

§Or tacrolimus + Mtx; a few patients received other regimens.

**Table 3.** *Results of Selected Studies of Immunosuppressive Therapy for Aplastic Anemia*

| Study | N* | Median Age (Range) | Treatment | Response | Relapse | Survival |
|---|---|---|---|---|---|---|
| Frickhofen et al [71,75]† | 43 | 32 (7-80) | ALG + MP + CSA | 70% at 4 months | 45% | 11 y OS = 58% |
| | 41 | 32 (2-67) | ALG + MP | 41% at 4 months | 30% | 11 y OS = 54% |
| Rosenfeld et al [74] | 122 | 35 | ATG + MP + CSA | 60% at 3 mths | 35% | 7 y OS = 55% |
| Kojima et al [104]† | 110 | 9 (1-18) | ATG + CSA + DAN‡ | 68% at 6 months | 22% | 3 y OS = 88% |

ALG indicates antilymphocyte globulin; MP, methylprednisolone; CSA, cyclosporine; DAN, danazol; OS, overall survival.
*Number of patients.
†Study included patients with nonsevere aplastic anemia.
‡± granulocyte colony-stimulating factor.

entail greater toxicity. Thus, the challenge of transplantation in AA is to achieve high engraftment rates, whereas minimizing transplant-related morbidity and mortality. This has implications for every aspect of transplantation, as discussed in the next sections.

## Conditioning Regimen

If graft failure depends on residual host immune cells, intensifying the conditioning regimen should be beneficial. This was initially accomplished through the addition of radiation to conditioning. Indeed, radiation-containing conditioning regimens resulted in lower rates of graft failure, as documented in several series [19], including European Bone Marrow Transplant Registry (EBMT) and International Bone Marrow Transplant Registry (IBMT) studies [18,20,21]. Unfortunately, radiation is also associated with significant early and late toxicity, including secondary malignancies [22]. In fact, in the above studies, there was no survival advantage to total-body irradiation (TBI) or total lymphoid irradiation regimens; the advantage of lower graft failure rates was negated by a higher incidence of acute GVHD (aGVHD) and pulmonary toxicity [20,21].

This deadlock was broken with the introduction of antithymocyte globulin (ATG) in the conditioning regimen, pioneered by Smith and colleagues in Boston [23], and by the Seattle group (who initially studied the combination of cyclophosphamide and ATG as part of the conditioning regimen for third transplants in patients with GF [24], and applied this experience to second transplants [25], and thereafter to first transplants [26]). In a recent analysis of 81 patients receiving an MRD graft with Cy + ATG conditioning, excellent outcomes were reported, with rates of engraftment of 96%, aGVHD (grade II-IV) of 24%, and chronic GVHD (cGVHD) of 26% [27]. With median follow-up of almost a decade, 15-year overall survival (OS) was 88%. The benefit of Cy + ATG conditioning over Cy + radiation was further confirmed by a French retrospective study of 133 patients transplanted from matched siblings [28]. In this analysis, use of thoraco abdominal irradiation in combination with cyclophosphamide was associated with higher GVHD rates (both aGVHD and cGVHD) and lower OS, compared to use of Cy + ATG (55% versus 95%). In this series, as in the one above, patients conditioned with Cy + ATG had excellent long-term survival. Therefore, Cy (200 mg/m$^2$ divided in 4 daily doses) + ATG (90 mg/kg divided in 3 daily doses) is the current standard for MRD transplant conditioning.

The benefit of ATG also extends to alternative donor transplantation. In this setting, the rates of GF are higher, likely reflecting a host-versus-graft immune attack that is increased by the greater antigenic disparity (presumably at minor histocompatibility loci). This made it more difficult to eliminate radiation from the conditioning regimen [29]. Kojima and colleagues [30] conducted a retrospective study of 154 Japanese patients receiving alternative donor transplants for severe AA. They showed that survival was superior when ATG was used in the conditioning regimen (5-year OS of 61%-75% versus 24%-53%, which was significant in multivariable analysis). In a multicenter prospective study of 87 patients receiving grafts from alternative donors (62 matched unrelated donors (MUD) and 25 mismatched unrelated donors (MMUD)), Deeg and colleagues [31] attempted to define the optimal TBI dose (in combination with Cy and ATG) in this setting. GF rates remained acceptably low with a radiation dose as low as 200 cGy (1% for MUD and 12% for MMUD), and survival was not compromised by decreasing the total body irradiation (TBI) dose. In fact, for MUD allografts, survival was highest in the 200 cGy cohort (although this did not achieve statistical significance). This conclusion is supported by the study of Kojima et al [30] cited above, which demonstrated excellent survival (90%) for the small group of patients who received 500 cGy or less of radiation in addition to Cy and ATG. Based on those data, the combination of Cy 200 mg/m2, ATG 90 mg/kg, and TBI 200 cGy is a reasonable choice of conditioning regimen for alternative donor transplants. By extension, this could also be a reasonable conditioning regimen for heavily pretransfused patients undergoing MRD transplant. A high number of pretransplant transfusions has been associated with an increased incidence of GF [20], and those patients may therefore be more akin to those receiving an

alternative donor transplant (although this is certainly beyond the scope of available data).

Once again, the success of ATG in conditioning aplastic patients for HSCT is consistent with the immune hypothesis of AA. The antihematopoietic activity in AA appears to be largely T cell dependent, as evidenced by laboratory and clinical data [3]. Indeed, the standard of care for IST is the combination of a calcineurin inhibitor (cyclosporine or tacrolimus) and ATG [32], which are both T cell-targeting drugs. Thus, specifically targeting the T cell population during transplant conditioning should maximize efficacy and minimize toxicity, compared to the broadly myelotoxic effects of radiation. It is therefore not surprising that the newest agents used in transplantation conditioning, fludarabine and alemtuzumab, are also T cell targeting agents.

Accumulating evidence with fludarabine-containing conditioning regimens is encouraging and consistent across studies. Two multicenter EBMT studies have examined fludarabine's role in both MRD and alternative donor transplants. In a retrospective analysis of 45 adult patients receiving MRD grafts with various fludarabine-containing conditioning regimens [33], OS (with median follow-up of 21 months) was 77% for the patients receiving fludarabine, ATG, and low-dose (<200 mg/kg) cyclophosphamide (versus 24% for other regimens). Those results are not easy to interpret given the high patient and treatment heterogeneity (as this study included frontline and second-line transplants, marrow and peripheral blood grafts, and various GVHD prophylaxis regimens); fortunately, ongoing prospective studies should consolidate those promising results. A prospective EBMT study of 38 patients receiving alternative donor grafts with a radiation-free conditioning regimen of fludarabine, cyclophosphamide, and ATG showed reasonable engraftment (82% overall, 68% in adult patients), and a 73% 2-year OS (with short follow-up) [34]. aGVHD (grade II-IV) occurred in only 11% of patients, and cGVHD in 27%. Those early results compare favorably with those obtained with radiation-containing conditioning, are consistent with other reports in related and unrelated transplants [35-39], and warrant further studies to define the optimal fludarabine-based regimen.

The experience with alemtuzumab is more preliminary. A British study described the outcome of 33 patients receiving a MRD graft conditioned with cyclophosphamide and alemtuzumab [40]. Seventy-six percent of patients engrafted, 14% developed grade II-IV aGVHD, and 4% developed cGVHD; 5-year OS was 81%. The same group transplanted 7 young patients with a MUD graft conditioned with alemtuzumab, fludarabine, and cyclophosphamide [41]. All patients engrafted, with no grade III-IV aGVHD and 17% cGVHD. OS in this study was 71%. It should be noted that 4 of the 7 patients had congenital AA, including 3 with Fanconi anemia (who, as discussed below, require a different conditioning regimen from patients with acquired AA). Another study from Poland described 100% survival in 5 patients with severe AA transplanted from matched siblings after alemtuzumab, fludarabine, and melphalan conditioning [42]. Those results are promising and deserve further evaluation.

## GVHD Prophylaxis

Initially, GVHD prophylaxis after transplantation consisted of methotrexate (Mtx) alone. The usefulness of cyclosporine (CSA) was first suggested in the early 1980s by a retrospective study of 37 patients receiving mostly Cy-conditioned MRD transplants [43]. Twenty-four patients received CSA, and were compared to 14 historic controls who had received Mtx alone for GVHD prophylaxis. Engraftment rates were superior in the CSA-treated patients (92% versus 74%). OS was also higher (73% versus 56%), even though GVHD was actually more common in the CSA group. Interestingly, 3 patients in this study lost their graft after initially successful engraftment, concomitantly with the withdrawal of CSA. This pattern of late graft failure has been subsequently reported in other patients [44,45], in association with mixed chimerism. This suggests that the benefit of CSA in AA transplantation may be not so much in improving GVHD control but in suppressing the host immune system and thereby preventing early graft rejection. This illustrates once again the importance of T cell suppression in the treatment of AA.

The benefit of CSA for survival was confirmed in other retrospective studies [20,46], and in an influential prospective trial conducted by Storb and colleagues [47]. In this trial, 46 patients receiving an MRD transplant after Cy-only conditioning were randomized to Mtx with or without CSA for GVHD prophylaxis. Patients receiving combined therapy (CSA + Mtx) had a lower risk of aGVHD (18% versus 53%), similar engraftment rates, and an improved 2-year OS (82% versus 60%), which remained statistically significant in multivariate analysis. Another randomized trial conducted by Locatelli and colleagues [48] showed a similar survival benefit to combined CSA + Mtx treatment.

Since that time, no other regimen has shown clear superiority over the combination of calcineurin inhibitor (CSA or tacrolimus) and Mtx. There is only limited experience with T cell depletion in transplantation for AA. However, most of the data point to an increased risk of GF [20,49]; hence, this cannot be recommended outside the context of a clinical trial. There is also no compelling physiologic reason why T cell depletion should be useful in AA, where the fundamental goal is suppression of *recipient* T cell

function. Whether GVHD prophylaxis regimens using combinations of other T cell targeting agents, such as rapamycin or mycophenolate mofetil (MMF), can improve on the results with CSA + Mtx, remains to be determined. There are isolated reports that CSA + MMF prophylaxis is an acceptable regimen [37,50], but the experience is as of yet too limited to reliably compare this regimen to CSA + Mtx.

### Source of Stem Cells

Up to now, most transplantation studies in AA have used unmanipulated bone marrow as the stem cell source. In recent years, there has been an explosive increase in the use of peripheral blood stem cells (PBSC) in HSCT for other indications. The successful use of PBSC transplants in AA has been reported [51,52]. However, a retrospective EBMT study concluded that use of PBSC, despite providing faster engraftment, was associated with an increased incidence of cGVHD and a significantly lower 2-year survival compared to marrow grafts (67% versus 80%) [53]. This is reminiscent of the results using posttransplant buffy coat infusion in Seattle: even though this procedure increased engraftment rates, it was associated with a higher incidence of chronic GVHD and was ultimately abandoned [54]. At present, it therefore seems wise to use bone marrow grafts, at least outside of a clinical trial.

Umbilical cord blood (UCB) is also increasingly being used as a source of stem cells, as this allows the transplantation of patients without an HLA-matched donor. A group in China treated 9 young adults suffering from severe AA with umbilical transplants using cyclophosphamide and antilymphocyte globulin conditioning [55]. Seven of the 9 patients had some level of engraftment, evidenced by the presence of stable mixed chimerism. After a median follow-up of 32 months, survival was nearly 80%. Successful complete chimerism was reported in a pediatric patient after an umbilical cord graft [56]; stable engraftment was also achieved after UCB grafts in children with congenital AA in two other reports [57,58]. Umbilical cord transplants therefore appear to be an option for patients with AA who lack a suitable donor, but more experience is needed to determine the long-term outcome of this approach.

## SELECTION OF PATIENTS FOR UPFRONT TRANSPLANTATION

### The Challenge

Under the best circumstances, allogeneic transplantation can result in excellent outcomes. Long-term follow-up of young patients transplanted from matched related donors in several studies have documented a 10-year survival in excess of 80%, with most survivors having a normal performance status [27,59-61]. This seems to apply irrespective of the cause of AA (as long as it is acquired AA). Patients with a drug or viral trigger, or patients with pregnancy-associated AA, are usually included in studies of acquired AA, and with some exceptions of unclear significance [62,63], their outcome appears to be similar to that of patients with idiopathic acquired AA [6,60]. HSCT is also potentially curative for patients whose AA is associated with PNH, as first demonstrated in the 1970s [64,65] and subsequently confirmed in many small studies [66-68]; again, there is no evidence that those patients do worse after HSCT than their counterparts with AA alone.

Those numbers, however, do not apply to older patients or those without an HLA-matched sibling, who do significantly worse after transplantation [7]. Other adverse prognostic factors that have consistently emerged from multivariate analyses are omission of CSA from the GVHD prophylaxis regimen, earlier year of transplantation, longer time from diagnosis to transplantation, and gender mismatching [6,17,30,49,69,70]. Moreover, HSCT carries risks that, though not fatal, can nonetheless significantly affect quality of life. These include aGVHD and cGVHD (the latter affecting 20%-50% of long-term survivors [59,60]). Transplant survivors are also at increased risk of growth abnormalities (for children), infertility, cataracts, hypothyroidism [61], and secondary solid tumors (with a 20-year risk around 2%, or higher in patients who have received radiation) [22].

This must be weighed against the risks and benefits of IST. The standard of care for IST is currently the combination of CSA (or tacrolimus) and ATG (used here interchangeably with antilymphocyte globulin [ALG]), based largely on the results of a German randomized trial [71] (and the disappointing results of using instead high-dose cyclophosphamide with autologous recovery [72,73]). In Europe and the United States, this regimen has a response rate of 60%-65%, and is associated with long-term (7 to 11 years) OS around 55% [74,75]. Here, too, there is some prognostic heterogeneity, with younger patients [76] and those with less severe disease [76-78] having a superior outcome (although the prognostic role of disease severity has been questioned in other series [6]). Naturally, IST also carries significant risks. The rate of relapse is 35%-45%; and whereas around 2/3 of patients who relapse can respond to a second course of IST [79], this contributes to morbidity and mortality. More ominously, patients with AA treated with IST have a significant risk of developing late clonal abnormalities, including PNH, myelodysplastic syndrome (MDS), and acute myelogenous leukemia (AML). The latter two complications are the major cause of premature death for patients who survive beyond 3 years after IST. In large series of patients treated with IST,

the rate of development of PNH was 15%-20%, and of MDS/AML was 10%-20% [3,80]. Finally, patients treated with IST also are at increased risk for secondary solid tumors, with an incidence around 2%, similar to that after HSCT [81].

The foregoing highlights the challenge of choosing between IST and HSCT as frontline treatment for a given patient. The two modalities have very different toxicity profiles, and different prognostic factors. This is further complicated by the issue of historic trends. Indeed, the outcome of AA treatment since the 1970s has improved dramatically. This has been particularly true in the field of transplantation, with many large multicenter retrospective series documenting an absolute increase in OS over the last 20 years approaching 50% (see Figure 1) [6,17,18,46,82,83]; this trend has continued even after 1991 [84]. Many of the responsible factors have been previously discussed. Another factor that bears mention is improvement in supportive care, especially the change in transfusion practice. As mentioned above, heavily pretransfused patients have a worse outcome [20,21,30,82], presumably by allo-immunization in patients who already have anti-hematopoietic immune activity. Restricting pretransplantation transfusions, using leuko-poor, irradiated blood products, and using single-donor apheresis platelet units, are likely responsible for part of the improvement in engraftment rates and survival. Better anti-infectious agents, and the use of protective transplant environments [85], also improve the survival of leukopenic patients and thereby blunt the impact of graft failure, as more patients can now survive to a second transplant.

Improvements in IST over time have also occurred, although they are perhaps slightly less pronounced. The trial that proved the value of today's standard of care (CSA + ATG) was published in 1991; no regimen has since improved on those results, and even that regimen was not proved to significantly increase OS compared to ATG alone [75]. The differing time trends in the outcome of IST and HSCT make older studies or studies with a long time-span less useful when comparing the two modalities, and clinicians must therefore rely on recent studies that cannot capture long-term effects of therapy.

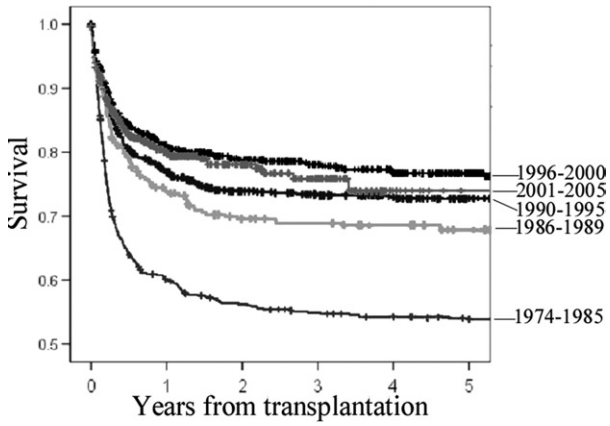


**Figure 1.** Survival after matched sibling transplantation for severe aplastic anemia, by year of transplantation, from the European Bone Marrow Transplant registry (courtesy of Jakob Passweg, on behalf of the Working Party Aplastic Anemia of the EBMT).

In the remainder of this review, we discuss several clinical scenarios, and in each case attempt to answer the question of upfront treatment choice. We will proceed in order of controversy, starting with situations where the answer is clear, and moving into areas where no right answer exists and where we can only offer our perspective and practice, understanding that it is impossible to answer the question with any degree of certainty.

## Congenital AA

The defects leading to inherited marrow failure syndromes are not autoimmune, as they are in acquired AA. Therefore, those patients will not respond to IST, and HSCT is the only potentially curative procedure. For patients with Fanconi anemia (FA), survival following MRD HSCT may approach that of patients transplanted for acquired AA (although patients with FA suffer from an increased incidence of GVHD and secondary malignancies [86]). Five-year OS of 60%-70% are typical, including in a large IBMT study [87]. As is the case for acquired AA, survival is worse after alternative donor HSCT [88].

The difficulty, then, is not so much in choosing the right treatment for patients with congenital AA, but in recognizing which patients belong to this category. In many cases, the diagnosis of an inherited marrow failure syndrome is made in childhood, based on the typical constellations of features that characterize each of the disorders. However, some patients do not develop marrow failure until adulthood; moreover, up to 1/3 of patients with FA (the most common inherited marrow failure syndrome) do not display obvious congenital anomalies [89]. The clinician approaching a patient with apparently acquired AA must therefore diligently search for clues to a congenital syndrome. In the case of FA, those include skin pigmentation abnormalities (most classically café-au-lait spots), hearing defects, macrocytosis, or solid tumors occurring at an unusually young age [90]; similarly, nail malformations, a reticular rash, oral leukoplakia, osteoporosis, or pulmonary fibrosis may suggest the diagnosis of dyskeratosis congenital (DKC); whereas exocrine pancreatic insufficiency may be a clue to the Schwachman-Blackfan-Diamond syndrome [2]. Because the telltale abnormalities may not be present in the patient but in a family member, a thorough family history is essential. Once a diagnosis of congenital AA is suspected, the appropriate diagnostic workup can be

pursued. For FA, chromosomal breakage testing can be performed using mitomycin C or diepoxybutane. Of note, a minority of FA patients display somatic mosaicism, in which case diagnosis requires testing of cultured skin fibroblasts rather than hematopoietic cells [91]. For the other inherited marrow failure syndromes, sequencing of the relevant genes must be done.

The other important implication of a diagnosis of congenital AA relates to the choice of conditioning regimen for transplantation. Patients with FA, who harbor defects in DNA repair, are exquisitely sensitive to DNA-damaging agents, so much so that standard conditioning regimens are lethal. Dose reductions (usually 70%-90% for cyclophosphamide and 50%-75% for radiation) are therefore essential. Fludarabine may also be useful in the conditioning regimen to make up for the dose reduction in Cy and TBI [92,93].

### Nonsevere AA

Severe AA is defined as marrow cellularity below 25% with at least 2 among: absolute neutrophil count (ANC) $<0.5 \times 10^9$/L, platelets $<20 \times 10^9$/L, and absolute reticulocyte count $<20 \times 10^9$/L. Very severe AA is the same except that ANC must be below $0.2 \times 10^9$/L [94]. Although there may be some prognostic difference between severe and very severe AA when treated with IST, as discussed above (if not when treated with HSCT [7,77]), it is clear that patients without at least severe AA are best not transplanted, and treated instead with observation, androgens, or IST. Their disease may remain mild or moderate and may even remit, and their survival (without HSCT) is in excess of 90% [95].

### Syngeneic Transplantation

For the few patients lucky enough to have an available identical twin donor, the outlook is extremely favorable. Because the cure of AA does not require a graft-versus-tumor effect, syngeneic transplants are ideal, as they minimize the risk of graft failure and eliminate that of GVHD. Hinterberger et al. [96] analyzed a cohort of 40 patients who received a syngeneic graft between 1964 and 1992, comparing those who received an unconditioned graft with those who were conditioned with cyclophosphamide-based regimens. The use of conditioning significantly improved engraftment rates (64% versus 30%), again consistent with the immune hypothesis of acquired AA; however, there was no significant difference in OS between the patients who received conditioning prior to the first transplantation attempt (with a 10-year OS of 70%) and those who did not (with a 10-year OS of 87%). This implies a high salvage rate for patients with graft failure after an unconditioned syngeneic graft; as those patients are spared both conditioning regimen toxicity and GVHD, they can more easily be supported until a subsequent conditioned transplant. In more recent series, syngeneic transplants are associated with a survival around 90% [6]. In this case, as with matched sibling donor, radiation as part of the conditioning regimen is not needed, and cyclophosphamide-alone conditioning should be sufficient.

### Matched Sibling Donor Transplantation

The current recommendation for patients with an available matched sibling donor, as proposed by Bacigalupo and colleagues [6], is to transplant patients younger than 40 and treat patients older than 40 with IST (reserving HSCT for relapsing or refractory patients). This distinction is based on the favorable outcomes with MRD HSCT in young patients, and the adverse prognostic impact of advancing age. However, there may be a rationale for using an older age cutoff in this case. As discussed above, the outcomes of MRD HSCT have improved markedly in the last 20 years. The benchmarks for HSCT outcomes should therefore be based on patients transplanted after 1990. In a retrospective multicenter EBMT analysis, OS after HSCT for patients over 40 years of age transplanted after 1990 was 54% [6]. It must be noted that 14% of patients in this study received a graft from alternative donors. Moreover, not all patients were transplanted upfront, and the outcome of HSCT is worse for patients who have previously received IST [18,63]. Therefore, the true OS after MRD HSCT in this age group is likely higher than 54%.

The most directly comparable numbers using IST come from another EBMT analysis of older patients [76]. In this study, the 5-year OS for patients aged 50-59 treated after 1990 was 60%. Although the 2 numbers (54% and 60%) are very comparable, several issues should be kept in mind. First, HSCT has the important advantage of avoiding the risk of late clonal diseases (12% at 10 years in the above IST study). Indeed, survival curves for patients treated with IST do not reach a plateau as clearly as the corresponding curves after HSCT, and there appears to be up to a 10% absolute decrease in survival between 5 and 10 years after IST in studies with long-term follow-up [75,77]. Second, patients in their 50s are close to the cutoff age for transplantation. If they are treated with an attempt at IST and relapse after a few years, they may have lost the opportunity for transplantation.

Given this, it may be reasonable to consider transplantation for first-line therapy in patients with severe AA up to the age of 55 or so when a matched sibling donor is available. For patients between the ages of 40 and 55, for whom the true difference in outcome between HSCT and IST is likely small, other prognostic considerations should weigh in. Patients with very severe AA (ANC below $0.2 \times 10^9$/L), as previ-

ously mentioned, appear to do worse than those with severe AA (ANC between 0.2 and 0.5 $\times$ $10^9$/L) when treated with IST, but not when transplanted [7,76-78,90]. Therefore, transplantation may be preferable for those patients. In contrast, patients with associated PNH clones (even if only apparent on flow cytometric studies) may do better after IST than patients with AA alone [97,98]. IST may therefore be the treatment of choice in this case. The unavailability of a gender-matched donor may also militate in favor of IST [70].

### MUD Transplantation

The current recommendation is to treat all patients without an available MRD with at least 2 attempts at IST prior to resorting to transplantation [6], based on the poorer results of MUD compared to MRD HSCT. Here also, our own perspective is more biased in favor of transplantation. Indeed, transplantation from alternative donors has benefited from the same improvements over time as has transplantation from matched siblings, even though the improvement in survival has been less dramatic. In a recent IBMT retrospective study of patients transplanted between 1988 and 1994, Passweg and colleagues [99] reported a 5-year OS after MUD transplantation of 39%, with age over 21 years and poor performance status being adverse risk factors for survival. However, more recent prospective experiences have demonstrated superior results. An EBMT trial of 38 children and young adults using fludarabine + Cy + ATG conditioning reported a 2-year OS of 74%, and 84% for patients under the age of 18 [34]. A recent comparison of alternative and matched related donors for pediatric transplants found no difference in OS [100]. Moreover, in the study of Deeg and colleagues [31] on TBI deescalation cited above, which was based on patients transplanted between 1994 and 2004, the survival after MUD HSCT was 61% (66% for those patients conditioned with 200 cGy TBI), and 73% for patients under the age of 21. The difference in outcomes between the study of Passweg and colleagues and the other cited studies might be explained by the quality of HLA typing. In Passweg et al's study, most patients were typed at low resolution, whereas in the others molecular typing was performed. This is significant because the outcome with mismatched unrelated donors is worse than that with matched unrelated donors [30,31,49]. Transplantation using molecularly matched unrelated donors and modern conditioning regimens may therefore yield survivals of 60%-80% in young patients. The benefit of allele-level HLA matching has recently been confirmed in a retrospective French study, in which young patients with fully HLA-matched unrelated donors had a 5-year survival of 78% [101]. It must also be remembered that, because of the historically poor outcomes with alternative donor transplants, patients receiving MUD transplants in clinical trials are patients who have previously failed at least one, and usually more courses of IST. In the study of Deeg et al [31], for example, patients had received a median of 3 courses of IST. The literature on MRD transplants has repeatedly demonstrated that longer time to HSCT and prior immunosuppression are adverse risk factors [21,30,49,63,102]. Therefore, the 60%-80% survival mentioned above could even underestimate the true survival of young patients transplanted with allele-level MUD allografts as front-line therapy.

Based on this, HSCT could be considered as first-line treatment for patients with severe AA under 21 years old (acknowledging the necessarily arbitrary nature of such a cutoff) for whom a molecularly matched unrelated donor is available. For older patients, or for patients without an allele-level MUD, IST is likely still the appropriate choice for frontline therapy.

### The Patient without an Available Matched Sibling or Unrelated Donor

As discussed above, outcomes after mismatched transplantation are poor, and the experience with umbilical cord grafts is still too sparse to justify its upfront use. Therefore, such patients should receive an attempt at IST (which can be repeated in case of failure or relapse). Only once IST options are exhausted can the risks of mismatched or UCB grafts be justified.

## CONCLUSION

The last 30 years have brought great progress both in our understanding of AA and in its treatment, so much so that the majority of patients can be cured with current therapies. Nonetheless, choosing the right treatment for an individual patient is a challenging task that begins with ruling out the presence of an inherited marrow failure syndrome. For patients with acquired severe AA, the choice of treatment depends principally on the patient's age and the availability of a matched donor. Assuming transplantation eligibility, we would consider this modality as front-line therapy for any patient with an available syngeneic donor, for patients up to the age of 55 or so with a matched sibling donor, and for patients up to the age of 21 or so with an allele-level HLA-matched unrelated donor. For all other patients, we would recommend IST as initial treatment. If history is a guide, the coming years will bring new developments in this field, requiring further reexamination of this question, and will continue to improve the prognosis of patients with this fascinating disease.

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